

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 January 2001 (18.01.2001)

PCT

(10) International Publication Number
WO 01/04325 A1

(51) International Patent Classification⁷: **C12N 15/54**,
15/62, C12P 13/08, C12Q 1/68, C12P 13/06, 13/22 //
(C12P 13/08, C12R 1:15)

View, Roscrea, County Tipperary (IE). **BURKE, Kevin**;
5, Greenfields Road, Newcastle, Galway, County Galway
(IE). **MÖCKEL, Bettina**; Benrodestrasse 35, D-40597
Düsseldorf (DE).

(21) International Application Number: PCT/EP00/06304

(22) International Filing Date: 5 July 2000 (05.07.2000)

(81) Designated States (*national*): AU, BR, CA, CN, HU, ID,
JP, KR, MX, PL, RU, SK, UA, ZA.

(25) Filing Language: English

(84) Designated States (*regional*): European patent (AT, BE,
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,
NL, PT, SE).

(26) Publication Language: English

(30) Priority Data:
60/142,915 9 July 1999 (09.07.1999) US
09/531,266 20 March 2000 (20.03.2000) US

Published:

- With international search report.
- Before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments.
- With (an) indication(s) in relation to deposited biological
material furnished under Rule 13bis separately from the
description.

(71) Applicants: **DEGUSSA-HÜLS AKTIENGE-
SELLSCHAFT** [DE/DE]; D-60287 Frankfurt am
Main (DE). **NATIONAL UNIVERSITY OF IRELAND**
[IE/IE]; Galway (IE).

(72) Inventors: **DUNICAN, L., K.** (deceased). **MC-
CORMACK, Ashling**; Moate Road, Athlone, County
Westmeath (IE). **STAPELTON, Cliona**; 27, Railway

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 01/04325 A1

(54) Title: NUCLEOTIDE SEQUENCES FOR THE TAL GENE

(57) Abstract: The invention relates to an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence chosen from the group consisting of a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4, b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70 % to the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4, c) polynucleotide which is complementary to the polynucleotides of a) or b) and, d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequences of a), b) or c) and a process for the preparation of L-amino acids, which comprises carrying out the following steps: a) fermentation of the desired L-amino acid-producing bacteria in which at least the tal gene is amplified, b) concentration of the desired product in the medium or in the cells of the bacteria and c) isolation of the L-amino acid.

NUCLEOTIDE SEQUENCES FOR THE TAL GENE

The invention provides nucleotide sequences which code for the tal gene and a process for the fermentative preparation of amino acids, in particular L-lysine, L-threonine, L-
5 isoleucine and L-tryptophan, using coryneform bacteria in which the tal gene is amplified.

Prior art --

Amino acids, in particular L-lysine, are used in human
medicine and in the pharmaceuticals industry, but in
10 particular in animal nutrition.

It is known that amino acids are prepared by fermentation by strains of coryneform bacteria, in particular Corynebacterium glutamicum. Because of their great
importance, work is constantly being undertaken to improve
15 the preparation processes. Improvements to the processes can relate to fermentation measures, such as e. g. stirring and supply of oxygen, or the composition of the nutrient media, such as e. g. the sugar concentration during the fermentation, or the working up to the product form by
20 e. g. ion exchange chromatography, or the intrinsic output properties of the microorganism itself.

Methods of mutagenesis, selection and mutant selection are used to improve the output properties of these microorganisms. Strains which are resistant to
25 antimetabolites, such as e. g. the lysine analogue S-(2-aminoethyl)-cysteine, or are auxotrophic for metabolites of regulatory importance and produce L-amino acids, such as e. g. L-lysine, are obtained in this manner.

Methods of the recombinant DNA technique have also been
30 employed for some years for improving the strain of Corynebacterium strains which produce amino acids, by amplifying individual amino acid biosynthesis genes and investigating the effect on the amino acid production.

Review articles in this context are to be found, inter alia, in Kinoshita ("Glutamic Acid Bacteria", in: Biology of Industrial Microorganisms, Demain and Solomon (Eds.), Benjamin Cummings, London, UK, 1985, 115-142), Hilliger 5 (BioTec 2, 40-44 (1991)), Eggeling (Amino Acids 6:261-272 (1994)), Jetten and Sinskey (Critical Reviews in Biotechnology 15, 73-103 (1995)) and Sahm et al. (Annals of the New York Academy of Science 782, 25-39 (1996)).

The importance of the pentose phosphate cycle for the 10 biosynthesis and production of amino acids, in particular L-lysine, by coryneform bacteria is the subject of numerous efforts among experts.

Thus Oishi and Aida (Agricultural and Biological Chemistry 29, 83-89 (1965)) report on the "hexose monophosphate 15 shunt" of *Brevibacterium ammoniagenes*. Sugimoto and Shio (Agricultural and Biological Chemistry 51, 101-108 (1987)) report on the regulation of glucose 6-phosphate dehydrogenase in *Brevibacterium flavum*.

Object of the invention

20 The inventors had the object of providing new measures for improved fermentative preparation of amino acids, in particular L-lysine, L-threonine, L-isoleucine and L-tryptophan.

Description of the invention

25 Amino acids, in particular L-lysine, are used in human medicine, in the pharmaceuticals industry and in particular in animal nutrition. There is therefore a general interest in providing new improved processes for the preparation of amino acids, in particular L-lysine.

30 When L-lysine or lysine are mentioned in the following, not only the base but also the salts, such as e. g. lysine

monohydrochloride or lysine sulfate, are also meant by this.

The invention provides an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence
5 chosen from the group consisting of

- a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4,
- 10 b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4,
- c) polynucleotide which is complementary to the
15 polynucleotides of a) or b) and
- d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c).

The invention also provides the polynucleotide as claimed
20 in claim 1, this preferably being a DNA which is capable of replication, comprising:

- (i) a nucleotide sequence chosen from the group consisting of SEQ ID NO. 1 and SEQ ID NO. 3 or
- (ii) at least one sequence which corresponds to
25 sequence (i) within the range of the degeneration of the genetic code, or
- (iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally
- 30 (iv) sense mutations of neutral function in (i).

The invention also provides

a polynucleotide as claimed in claim 4, comprising one of the nucleotide sequences as shown in SEQ ID NO. 1 and SEQ ID NO. 3,

5 a polynucleotide as claimed in claim 5, which codes for a polypeptide which comprises the amino acid sequence as shown in SEQ ID NO. 2 and SEQ ID NO. 4,

a vector containing the polynucleotide as claimed in claim 1,

10 and coryneform bacteria, serving as the host cell, which contain the vector.

The invention also provides polynucleotides which substantially comprise a polynucleotide sequence, which are obtainable by screening by means of hybridization of a
15 corresponding gene library, which comprises the complete gene with the polynucleotide sequence corresponding to SEQ ID NO. 1 or SEQ ID NO. 3, with a probe which comprises the sequence of the polynucleotide mentioned, according to

SEQ ID NO. 1 or SEQ ID NO. 3 or a fragment thereof, and
20 isolation of the DNA sequence mentioned.

Polynucleotide sequences according to the invention are suitable as hybridization probes for RNA, cDNA and DNA, in order to isolate, in the full length, cDNA which code for transaldolase and to isolate those cDNA or genes which have
25 a high similarity of sequence with that of the transaldolase gene.

Polynucleotide sequences according to the invention are furthermore suitable as primers for the preparation of DNA of genes which code for transaldolase by the polymerase
30 chain reaction (PCR).

Such oligonucleotides which serve as probes or primers comprise at least 30, preferably at least 20, especially preferably at least 15 successive nucleotides.

Oligonucleotides which have a length of at least 40 or 50 nucleotides are also suitable.

"Isolated" means separated out of its natural environment.

"Polynucleotide" in general relates to polyribonucleotides and polydeoxyribonucleotides, it being possible for these to be non-modified RNA or DNA or modified RNA or DNA.

10 "Polypeptides" is understood as meaning peptides or proteins which comprise two or more amino acids bonded via peptide bonds.

The polypeptides according to the invention include a polypeptide according to SEQ ID NO. 2 or SEQ ID NO. 4, in particular those with the biological activity of transaldolase, and also those which are identical to the extent of at least 70 % to the polypeptide according to SEQ ID NO. 2 or SEQ ID NO. 4, and preferably are identical to the extent of at least 80% and in particular to the extent of at least 90 % to 95 % to the polypeptide according to SEQ ID NO. 2 or SEQ ID NO. 4, and have the activity mentioned.

The invention also provides a process for the fermentative preparation of amino acids, in particular L-lysine, L-threonine, L-isoleucine and L-tryptophan, using coryneform bacteria which in particular already produce an amino acid, and in which the nucleotide sequences which code for the tal gene are amplified, in particular over-expressed.

The term "amplification" in this connection describes the increase in the intracellular activity of one or more enzymes in a microorganism which are coded by the corresponding DNA, for example by increasing the number of copies of the gene or genes, using a potent promoter or

using a gene which codes for a corresponding enzyme having a high activity, and optionally combining these measures.

The microorganisms which the present invention provides can prepare L-amino acids, in particular L-lysine, from
5 glucose, sucrose, lactose, fructose, maltose, molasses, starch, cellulose or from glycerol and ethanol. They can be representatives of coryneform bacteria, in particular of the genus *Corynebacterium*. Of the genus *Corynebacterium*, there may be mentioned in particular the species
10 *Corynebacterium glutamicum*, which is known among experts for its ability to produce L-amino acids.

Suitable strains of the genus *Corynebacterium*, in particular of the species *Corynebacterium glutamicum*, are, for example, the known wild-type strains

- 15 *Corynebacterium glutamicum* ATCC13032
- Corynebacterium acetoglutamicum* ATCC15806
- Corynebacterium acetoacidophilum* ATCC13870
- Corynebacterium thermoaminogenes* FERM BP-1539
- Corynebacterium melassecola* ATCC17965
- 20 *Brevibacterium flavum* ATCC14067
- Brevibacterium lactofermentum* ATCC13869 and
- Brevibacterium divaricatum* ATCC14020

and L-lysine-producing mutants or strains prepared therefrom, such as, for example

- 25 *Corynebacterium glutamicum* FERM-P 1709
- Brevibacterium flavum* FERM-P 1708
- Brevibacterium lactofermentum* FERM-P 1712
- Corynebacterium glutamicum* FERM-P 6463
- Corynebacterium glutamicum* FERM-P 6464 and
- 30 *Corynebacterium glutamicum* ATCC13032
- Corynebacterium glutamicum* DM58-1
- Corynebacterium glutamicum* DSM12866.

and L-threonine-producing mutants or strains prepared therefrom, such as, for example

- Corynebacterium glutamicum ATCC21649
- Brevibacterium flavum BB69
- 5 Brevibacterium flavum DSM5399
- Brevibacterium lactofermentum FERM-BP 269
- Brevibacterium lactofermentum TBB-10

and L-isoleucine-producing mutants or strains prepared therefrom, such as, for example

- 10 Corynebacterium glutamicum ATCC 14309
- Corynebacterium glutamicum ATCC 14310
- Corynebacterium glutamicum ATCC 14311
- Corynebacterium glutamicum ATCC 15168
- Corynebacterium ammoniagenes ATCC 6871

15 and L-tryptophan-producing mutants or strains prepared therefrom, such as, for example

- Corynebacterium glutamicum ATCC21850 and
- Corynebacterium glutamicum KY9218 (pKW9901)

The inventors have succeeded in isolating the new tal gene
20 of *C. glutamicum* which codes for transaldolase (EC 2.2.1.2).

To isolate the tal gene or also other genes of *C. glutamicum*, a gene library of this microorganism is first set up in *E. coli*. The setting up of gene libraries is
25 described in generally known textbooks and handbooks. The textbook by Winnacker: Gene und Klone, Eine Einführung in die Gentechnologie [Genes and Clones, An Introduction to Genetic Engineering] (Verlag Chemie, Weinheim, Germany, 1990) or the handbook by Sambrook et al.: Molecular
30 Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1989) may be mentioned as an example. A well-known gene library is that of the *E. coli* K-12 strain W3110 set

up in λ vectors by Kohara et al. (Cell 50, 495-508 (1987)). Bathe et al. (Molecular and General Genetics, 252:255-265, 1996) describe a gene library of *C. glutamicum* ATCC13032, which was set up with the aid of the cosmid vector SuperCos I (Wahl et al., 1987, Proceedings of the National Academy of Sciences USA, 84:2160-2164) in the *E. coli* K-12 strain NM554 (Raleigh et al., 1988, Nucleic Acids Research 16:1563-1575). Börmann et al. (Molecular Microbiology 6(3), 317-326) (1992)) in turn describe a gene library of *C. glutamicum* ATCC13032 using the cosmid pH79 (Hohn and Collins, Gene 11, 291-298 (1980)). O'Donohue (The Cloning and Molecular Analysis of Four Common Aromatic Amino Acid Biosynthetic Genes from *Corynebacterium glutamicum*. Ph.D. Thesis, National University of Ireland, Galway, 1997) describes the cloning of *C. glutamicum* genes using the λ Zap expression system described by Short et al. (Nucleic Acids Research, 16: 7583). To prepare a gene library of *C. glutamicum* in *E. coli* it is also possible to use plasmids such as pBR322 (Bolivar, Life Sciences, 25, 807-818 (1979)) or pUC9 (Vieira et al., 1982, Gene, 19:259-268). Suitable hosts are, in particular, those *E. coli* strains which are restriction- and recombination-defective. An example of these is the strain DH5 α mc^r, which has been described by Grant et al. (Proceedings of the National Academy of Sciences USA, 87 (1990) 4645-4649). The long DNA fragments cloned with the aid of cosmids can then in turn be subcloned and subsequently sequenced in the usual vectors which are suitable for sequencing, such as is described e. g. by Sanger et al. (Proceedings of the National Academy of Sciences of the United States of America, 74:5463-5467, 1977).

The DNA sequences obtained can then be investigated with known algorithms or sequence analysis programs, such as e. g. that of Staden (Nucleic Acids Research 14, 217-232(1986)), the GCG program of Butler (Methods of Biochemical Analysis 39, 74-97 (1998)) the FASTA algorithm

of Pearson and Lipman (Proceedings of the National Academy of Sciences USA 85,2444-2448 (1988)) or the BLAST algorithm of Altschul et al. (Nature Genetics 6, 119-129 (1994)) and compared with the sequence entries which exist in databanks
5 accessible to the public. Databanks for nucleotide sequences which are accessible to the public are, for example, that of the European Molecular Biologies Laboratories (EMBL, Heidelberg, Germany) of that of the National Center for Biotechnology Information (NCBI,
10 Bethesda, MD, USA).

The invention provides the new DNA sequence from C.glutamicum which contains the DNA section which codes for the tal gene, shown as SEQ ID NO 1 and SEQ ID NO 3. The amino acid sequence of the corresponding protein has
15 furthermore been derived from the present DNA sequence using the methods described above. The resulting amino acid sequence of the tal gene product is shown in SEQ ID NO 2 and SEQ ID NO 4.

A gene library produced in the manner described above can
20 furthermore be investigated by hybridization with nucleotide probes of known sequence, such as, for example, the zwf gene (JP-A-09224661). The cloned DNA of the clones which show a positive reaction in the hybridization is sequenced in turn to give on the one hand the known
25 nucleotide sequence of the probe employed and on the other hand the adjacent new DNA sequences.

Coding DNA sequences which result from SEQ ID NO 3 by the degeneracy of the genetic code are also a constituent of the invention. In the same way, DNA sequences which
30 hybridize with SEQ ID NO 3 or parts of or SEQ ID NO 3 are a constituent of the invention. Conservative amino acid exchanges, such as e. g. exchange of glycine for alanine or of aspartic acid for glutamic acid in proteins, are furthermore known among experts as "sense mutations" which
35 do not lead to a fundamental change in the activity of the

protein, i.e. are of neutral function. It is furthermore known that changes on the N and/or C terminus of a protein cannot substantially impair or can even stabilize the function thereof. Information in this context can be found
5 by the expert, inter alia, in Ben-Bassat et al. (Journal of Bacteriology 169:751-757 (1987)), in O'Regan et al. (Gene 77:237-251 (1989)), in Sahin-Toth et al. (Protein Sciences 3:240-247 (1994)), in Hochuli et al. (Bio/Technology 6:1321-1325 (1988)) and in known textbooks of genetics and
10 molecular biology. Amino acid sequences which result in a corresponding manner from SEQ ID NO 2 or SEQ ID NO 4 are also a constituent of the invention.

In the same way, DNA sequences which hybridize with or SEQ ID NO 3 or parts of or SEQ ID NO 3 are a constituent of the
15 invention. Finally, DNA sequences which are prepared by the polymerase chain reaction (PCR) using primers which result from SEQ ID NO 3 are a constituent of the invention. Such oligonucleotides typically have a length of at least 15 nucleotides.

20 Instructions for identifying DNA sequences by means of hybridization can be found by the expert, inter alia, in the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl et al. (International Journal
25 of Systematic Bacteriology (1991) 41: 255-260). Instructions for amplification of DNA sequences with the aid of the polymerase chain reaction (PCR) can be found by the expert, inter alia, in the handbook by Gait: Oligonukleotide synthesis: a practical approach (IRL Press,
30 Oxford, UK, 1984) and in Newton and Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Germany, 1994).

The inventors have found that coryneform bacteria produce amino acids in an improved manner after over-expression of the tal gene.

To achieve an over-expression, the number of copies of the corresponding genes can be increased, or the promoter and regulation region or the ribosome binding site upstream of the structural gene can be mutated. Expression cassettes
5 which are incorporated upstream of the structural gene act in the same way. By inducible promoters, it is additionally possible to increase the expression in the course of fermentative L-amino acid production. The expression is likewise improved by measures to prolong the life of the m-
10 RNA. Furthermore, the enzyme activity is also increased by preventing the degradation of the enzyme protein. The genes or gene constructs can either be present in plasmids with a varying number of copies, or can be integrated and amplified in the chromosome. Alternatively, an over-
15 expression of the genes in question can furthermore be achieved by changing the composition of the media and the culture procedure.

Instructions in this context can be found by the expert, inter alia, in Martin et al. (Bio/Technology 5, 137-146
20 (1987)), in Guerrero et al. (Gene 138, 35-41 (1994)), Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)), in Eikmanns et al. (Gene 102, 93-98 (1991)), in European Patent Specification EPS 0 472 869, in US Patent 4,601,893, in Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991)), in
25 Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)), in LaBarre et al. (Journal of Bacteriology 175, 1001-1007 (1993)), in Patent Application WO 96/15246, in Malumbres et al. (Gene 134, 15 - 24 (1993)), in Japanese Laid-Open Specification JP-A-10-
30 229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996)) and in known textbooks of genetics and molecular biology.

By way of example, the tal gene according to the invention
35 was over-expressed with the aid of plasmids.

Suitable plasmids are those which are replicated in coryneform bacteria. Numerous known plasmid vectors, such as e. g. pZ1 (Menkel et al., Applied and Environmental Microbiology (1989) 64: 549-554), pEKEx1 (Eikmanns et al., 5 Gene 102:93-98 (1991)) or pHS2-1 (Sonnen et al., Gene 107:69-74 (1991)) are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, such as e. g. those based on pCG4 (US-A 4,489,160), or pNG2 (Serwold-Davis et al., FEMS Microbiology Letters 66, 119-124 10 (1990)), or pAG1 (US-A 5,158,891), can be used in the same manner.

Plasmid vectors which are furthermore suitable are also those with the aid of which the process of gene amplification by integration into the chromosome can be 15 used, as has been described, for example, by Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)) for duplication or amplification of the hom-thrB operon. In this method, the complete gene is cloned in a plasmid vector which can replicate in a host (typically E. 20 coli), but not in C. glutamicum. Possible vectors are, for example, pSUP301 (Simon et al., Bio/Technology 1, 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., Gene 145, 69-73 (1994)), pGEM-T (Promega corporation, Madison, WI, USA), pCR2.1-TOPO (Shuman (1994). Journal of Biological 25 Chemistry 269:32678-84; US-A 5,487,993), pCR@Blunt (Invitrogen, Groningen, Holland; Bernard et al., Journal of Molecular Biology, 234: 534-541 (1993)), pEM1 (Schrumpf et al, 1991, Journal of Bacteriology 173:4510-4516) or pBGS8 (Spratt et al., 1986, Gene 41: 337-342). The plasmid vector 30 which contains the gene to be amplified is then transferred into the desired strain of C. glutamicum by conjugation or transformation. The method of conjugation is described, for example, by Schäfer et al. (Applied and Environmental Microbiology 60, 756-759 (1994)). Methods for 35 transformation are described, for example, by Thierbach et al. (Applied Microbiology and Biotechnology 29, 356-362

(1988)), Dunican and Shivnan (Bio/Technology 7, 1067-1070 (1989)) and Tauch et al. (FEMS Microbiological Letters 123, 343-347 (1994)). After homologous recombination by means of a "cross over" event, the resulting strain contains at least two copies of the gene in question.

An example of a plasmid vector with the aid of which the process of amplification by integration can be carried out is pSUZ1, which is shown in Figure 1. Plasmid pSUZ1 consists of the E. coli vector pBGS8 described by Spratt et al. (Gene 41: 337-342(1986)), into which the tal gene has been incorporated.

In addition, it may be advantageous for the production of amino acids to amplify or over-express one or more enzymes of the particular biosynthesis pathway, of glycolysis, of anaplerosis, of the pentose phosphate pathway or of amino acid export, in addition to the tal gene.

Thus, for example, for the preparation of L-amino acids, in particular L-lysine, one or more genes chosen from the group consisting of

- 20 • the dapA gene which codes for dihydrodipicolinate synthase (EP-B 0 197 335),
- the lysC gene which codes for a feed back resistant aspartate kinase (Kalinowski et al. (1990), Molecular and General Genetics 224: 317-324),
- 25 • the gap gene which codes for glycerolaldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the pyc gene which codes for pyruvate carboxylase (DE-A-198 31 609),
- 30 • the mqo gene which codes for malate:quinone oxidoreductase (Molenaar et al., European Journal of Biochemistry 254, 395-403 (1998)),

- the tkt gene which codes for transketolase (accession number AB023377 of the databank of European Molecular Biologies Laboratories (EMBL, Heidelberg, Germany)),
- the gnd gene which codes for 6-phosphogluconate
5 dehydrogenase (JP-A-9-224662),
- the zwf gene which codes for glucose 6-phosphate dehydrogenase (JP-A-9-224661),
- the lysE gene which codes for lysine export (DE-A-195 48 222),
- 10 • the zwf gene (DE 199 59 328.0; DSM 13115),
- the eno gene which codes for enolase (DE: 19947791.4),
- the devB gene,
- the opcA gene (DSM 13264)

can be amplified, preferably over-expressed, at the same
15 time.

Thus, for example, for the preparation of L-threonine, one or more genes chosen from the group consisting of

- at the same time the hom gene which codes for homoserine dehydrogenase (Peoples et al., Molecular Microbiology 2,
20 63-72 (1988)) or the hom^{dr} allele which codes for a "feed back resistant" homoserine dehydrogenase (Archer et al., Gene 107, 53-59 (1991),
- the gap gene which codes for glycerolaldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology
25 174:6076-6086),
- the pyc gene which codes for pyruvate carboxylase (DE-A-198 31 609),

- the mgo gene which codes for malate:quinone oxidoreductase (Molenaar et al., European Journal of Biochemistry 254, 395-403 (1998)),
- the tkt gene which codes for transketolase (accession
5 number AB023377 of the databank of European Molecular
Biologies Laboratories (EMBL, Heidelberg, Germany)),
- the gnd gene which codes for 6-phosphogluconate dehydrogenase (JP-A-9-224662),
- the zwf gene which codes for glucose 6-phosphate
10 dehydrogenase (JP-A-9-224661),
- the thrE gene which codes for threonine export (DE 199 41
478.5; DSM 12840),
- the zwf gene (DE 199 59 328.0; DSM 13115),
- the eno gene which codes for enolase (DE: 19947791.4),
- 15 • the devB gene,
- the opcA gene (DSM 13264)

can be amplified, preferably over-expressed, at the same time.

It may furthermore be advantageous for the production of
20 amino acids to attenuate

- the pck gene which codes for phosphoenol pyruvate carboxykinase (DE 199 50 409.1 DSM 13047) and/or
- the pgi gene which codes for glucose 6-phosphate isomerase (US 09/396,478, DSM 12969), or
- 25 • the poxB gene which codes for pyruvate oxidase (DE 199 51 975.7; DSM 13114), or

- the *zwa2* gene (DE: 199 59 327.2; DSM 13113)

at the same time, in addition to the amplification of the *tal* gene.

In addition to over-expression of the *tal* gene it may
5 furthermore be advantageous for the production of amino acids to eliminate undesirable side reactions (Nakayama: "Breeding of Amino Acid Producing Micro-organisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

10 The microorganisms prepared according to the invention can be cultured continuously or discontinuously in the batch process (batch culture) or in the fed batch (feed process) or repeated fed batch process (repetitive feed process) for the purpose of production of L-amino acids. A summary of
15 known culture methods are described in the textbook by Chmiel (Bioprozeßtechnik 1. Einführung in die Bioverfahrenstechnik [Bioprocess Technology 1. Introduction to Bioprocess Technology (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und
20 periphere Einrichtungen [Bioreactors and Peripheral Equipment] (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must meet the requirements of the particular strains in a suitable manner. Descriptions of culture media for various microorganisms are contained
25 in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981). Sugars and carbohydrates, such as e. g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, such as
30 e. g. soya oil, sunflower oil, groundnut oil and coconut fat, fatty acids, such as e. g. palmitic acid, stearic acid and linoleic acid, alcohols, such as e. g. glycerol and ethanol, and organic acids, such as e. g. acetic acid, can be used as the source of carbon. These substances can be

used individually or as a mixture. Organic nitrogen-containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soya bean flour and urea, or inorganic compounds, such as ammonium sulphate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate, can be used as the source of nitrogen. The sources of nitrogen can be used individually or as a mixture. Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts can be used as the source of phosphorus. The culture medium must furthermore comprise salts of metals, such as e. g. magnesium sulfate or iron sulfate, which are necessary for growth. Finally, essential growth substances, such as amino acids and vitamins, can be employed in addition to the abovementioned substances. Suitable precursors can moreover be added to the culture medium. The starting substances mentioned can be added to the culture in the form of a single batch, or can be fed in during the culture in a suitable manner.

Basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia, or acid compounds, such as phosphoric acid or sulfuric acid, can be employed in a suitable manner to control the pH of the culture.

Antifoams, such as e. g. fatty acid polyglycol esters, can be employed to control the development of foam. Suitable substances having a selective action, such as e. g. antibiotics, can be added to the medium to maintain the stability of plasmids. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, such as e. g. air, are introduced into the culture. The temperature of the culture is usually 20°C to 45°C, and preferably 25°C to 40°C. Culturing is continued until a maximum of L-amino acid has formed. This target is usually reached within 10 hours to 160 hours.

The analysis of L-amino acids can be carried out by anion exchange chromatography with subsequent ninhydrin derivatization, as described by Spackman et al. (Analytical Chemistry, 30, (1958), 1190).

- 5 The following microorganism has been deposited at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) in accordance with the Budapest Treaty:
- 10 • *Escherichia coli* JM109/pSUZ1 as DSM 13263.

SEQ ID NO 1 also contains the new devB gene. The process according to the invention is used for fermentative preparation of amino acids.

The following figures are attached:

Figure 1: Map of the plasmid pSUZ1

The abbreviations and designations used have the following meaning.

- | | | |
|----|----------|---|
| 5 | lacZ: | segments of lacZ α gene fragment |
| | kan r: | kanamycin resistance |
| | tal: | transaldolase gene |
| | ori: | origin of replication of plasmid pBGS8 |
| | BclI: | cleavage site of restriction enzyme BclI |
| 10 | EcoRI: | cleavage site of restriction enzyme EcoRI |
| | HindIII: | cleavage site of restriction enzyme HindIII |
| | PstI: | cleavage site of restriction enzyme PstI |
| | SacI: | cleavage site of restriction enzyme SacI |

Examples

The following examples will further illustrate this invention. The molecular biology techniques, e.g. plasmid DNA isolation, restriction enzyme treatment, ligations, 5 standard transformations of *Escherichia coli* etc. used are, (unless stated otherwise), described by Sambrook et al., (Molecular Cloning. A Laboratory Manual (1989) Cold Spring Harbour Laboratories, USA).

Example 1

- 10 Preparation of a genomic cosmid gene library from
Corynebacterium glutamicum ATCC 13032

Chromosomal DNA from *Corynebacterium glutamicum* ATCC 13032 was isolated as described by Tauch et al. (1995, Plasmid 33:168-179) and partly cleaved with the restriction enzyme 15 Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Code no. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Code no. 1758250). The DNA of the cosmid 20 vector SuperCos1 (Wahl et al. (1987) Proceedings of the National Academy of Sciences USA 84:2160-2164), obtained from Stratagene (La Jolla, USA, Product Description SuperCos1 Cosmid Vector Kit, Code no. 251301) was cleaved with the restriction enzyme XbaI (Amersham Pharmacia, 25 Freiburg, Germany, Product Description XbaI, Code no. 27-0948-02) and likewise dephosphorylated with shrimp alkaline phosphatase. The cosmid DNA was then cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Code no. 27-0868-04). 30 The cosmid DNA treated in this manner was mixed with the treated ATCC13032 DNA and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product Description T4-DNA-Ligase, Code no. 27-0870-04). The ligation mixture was then packed in phages with the aid of

Gigapack II XL Packing Extracts (Stratagene, La Jolla, USA, Product Description Gigapack II XL Packing Extract, Code no. 200217). For infection of the E. coli strain NM554 (Raleigh et al. 1988, Nucleic Acid Research 16:1563-1575)

5 the cells were taken up in 10 mM MgSO₄ and mixed with an aliquot of the phage suspension. The infection and titering of the cosmid library were carried out as described by Sambrook et al. (1989, Molecular Cloning: A laboratory Manual, Cold Spring Harbor), the cells being plated out on

10 LB agar (Lennox, 1955, Virology, 1:190) with 100 µg/ml ampicillin. After incubation overnight at 37°C, recombinant individual clones were selected.

Example 2

Isolation and sequencing of the tal gene

15 The cosmid DNA of an individual colony was isolated with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product

20 Description Sau3AI, Product No. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Product No. 1758250). After separation by gel electrophoresis, the cosmid

25 fragments in the size range of 1500 to 2000 bp were isolated with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany). The DNA of the sequencing vector pZero-1, obtained from Invitrogen (Groningen, Holland, Product Description Zero Background Cloning Kit,

30 Product No. K2500-01) was cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Product No. 27-0868-04). The ligation of the cosmid fragments in the sequencing vector pZero-1 was carried out as described by Sambrook et al.

35 (1989, Molecular Cloning: A laboratory Manual, Cold Spring

Harbor), the DNA mixture being incubated overnight with T4 ligase (Pharmacia Biotech, Freiburg, Germany). This ligation mixture was then electroporated (Tauch et al. 1994, FEMS Microbiol Letters, 123:343-7) into the E. coli strain DH5 α MCR (Grant, 1990, Proceedings of the National Academy of Sciences U.S.A., 87:4645-4649) and plated out on LB agar (Lennox, 1955, Virology, 1:190) with 50 μ g/ml zeocin. The plasmid preparation of the recombinant clones was carried out with Biorobot 9600 (Product No. 900200, Qiagen, Hilden, Germany). The sequencing was carried out by the dideoxy chain-stopping method of Sanger et al. (1977, Proceedings of the National Academy of Sciences U.S.A., 74:5463-5467) with modifications according to Zimmermann et al. (1990, Nucleic Acids Research, 18:1067). The "RR dRhodamin Terminator Cycle Sequencing Kit" from PE Applied Biosystems (Product No. 403044, Weiterstadt, Germany) was used. The separation by gel electrophoresis and analysis of the sequencing reaction were carried out in a "Rotiphoresis NF Acrylamide/Bisacrylamide" Gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) with the "ABI Prism 377" sequencer from PE Applied Biosystems (Weiterstadt, Germany).

The raw sequence data obtained were then processed using the Staden program package (1986, Nucleic Acids Research, 14:217-231) version 97-0. The individual sequences of the pZero1 derivatives were assembled to a continuous contig. The computer-assisted coding region analysis were prepared with the XNIP program (Staden, 1986, Nucleic Acids Research, 14:217-231). Further analyses were carried out with the "BLAST search program" (Altschul et al., 1997, Nucleic Acids Research, 25:3389-3402), against the non-redundant databank of the "National Center for Biotechnology Information" (NCBI, Bethesda, MD, USA).

The nucleotide sequence obtained is shown in SEQ ID NO 1 and SEQ ID NO 3.

Example 3

Cloning of the tal gene

PCR was used to amplify DNA fragments containing the entire tal gene of *C. glutamicum* 13032 and flanking upstream and downstream regions. PCR reactions were carried out using oligonucleotide primers designed from the sequence as determined in Examples 1 and 2. Genomic DNA was isolated from *Corynebacterium glutamicum* ATCC13032 according to Heery and Dunican (Applied and Environmental Microbiology 59: 791-799 (1993)) and used as template. The tal primers used were:

fwd. primer: 5' GGT ACA AAG GGT CTT AAG 3'C

rev. primer: 5' GAT TTC ATG TCG CCG TTA 3'

PCR Parameters were as follows:

15 35 cycles
 95°C for 3 minutes
 94°C for 1 minute
 47°C for 1 minute
 72°C for 45 seconds
20 2.0 mM MgCl₂
 approximately 150-200 ng DNA template.

The PCR product obtained was cloned into the commercially available pGEM-T vector purchased from Promega Corp. (pGEM-T Easy Vector System 1, cat. no. A1360, Promega UK, Southampton, UK) using strain *E. coli* JM109 (Yanisch-Perron et al., Gene, 33: 103-119 (1985)) as a host. The entire tal gene was subsequently isolated from the pGEM T-vector on an Eco RI fragment and cloned into the lacZ α EcoRI site of the *E. coli* vector pBGS8 (Spratt et al., Gene 41(2-3): 337-342 (1986)). The restriction enzymes used were obtained from Boehringer Mannheim UK Ltd. (Bell Lane, Lewes East Sussex BN7 1LG, UK) and used according to manufacturer's instructions. *E. coli* JMI09 was then transformed with this ligation mixture and electrotransformants were selected on

Luria agar supplemented with isopropyl-thiogalactopyranoside (IPTG), 5-bromo-4-chloro-3-indolyl-galactopyranoside (XGAL) and kanamycin at concentrations of 1mM, 0.02% and 50 mg/l respectively. Plates were incubated
5 for twelve hours at 37°C. Plasmid DNA was isolated from one transformant, characterised by restriction enzyme analysis using Eco RI. This new construct was designated pSUZ 1.

Example 4

Preparation of the strain *Corynebacterium glutamicum*
10 DSM5715::pSUZ1

The strain DSM5715 was transformed with the plasmid pSUZ1 using the electroporation method described by Liebl et al., (FEMS Microbiology Letters, 53:299-303 (1989)). Selection of the transformants took place on LBHIS agar comprising
15 18.5 g/l brain-heart infusion broth, 0.5M sorbitol, 5 g/l Bacto-tryptone, 2.5 g/l Bacto-yeast extract, 5 g/l NaCl and 18 g/l Bacto-agar, which had been supplemented with 25 mg/l kanamycin. Incubation was carried out for 2 days at 33°C.

Since the vector pSUZ1 cannot replicate in the strain
20 DSM5715, only clones which show kanamycin resistance imparted by integration of pSUZ1 were able to grow.

The resulting integrant was called DSM5715::pSUZ1.

Example 5

Preparation of lysine

25 The *C. glutamicum* strain DSM5715/pSUZ1 obtained in Example 4 was cultured in a nutrient medium suitable for the production of L-lysine and the L-lysine content in the culture supernatant was determined.

For this, the strain was first incubated on an agar plate
30 with the corresponding antibiotic (brain-heart agar with kanamycin (25 mg/l)) for 24 hours at 33°C. Starting from

this agar plate culture, a preculture was seeded (10 ml medium in a 100 ml conical flask). The complete medium CgIII was used as the medium for the preculture.

Medium Cg III:

NaCl	2.5 g/l
Bacto-Peptide	10 g/l
Bacto-Yeast extract	10 g/l
Glucose (autoclaved separately)	2% (w/v)

The pH was brought to pH 7.4.

Kanamycin (25 mg/l) was added to this. The preculture was
5 incubated for 16 hours at 33°C at 240 rpm on a shaking machine. A main culture was seeded from this preculture such that the initial OD (660nm) of the main culture was 0.1. Medium MM was used for the main culture.

Medium MM:

CSL (corn steep liquor)	5 g/l
MOPS (morpholinopropanesulfonic acid)	20 g/l
Glucose (autoclaved separately)	50g/l
 (NH ₄) ₂ SO ₄	 25 g/l
KH ₂ PO ₄	0.1 g/l
MgSO ₄ * 7 H ₂ O	1.0 g/l
CaCl ₂ * 2 H ₂ O	10 mg/l
FeSO ₄ * 7 H ₂ O	10 mg/l

MnSO ₄ * H ₂ O	5.0mg/l
Biotin (sterile-filtered)	0.3 mg/l
Thiamine * HCl (sterile-filtered)	0.2 mg/l
L-Leucine	0.1 g/l
CaCO ₃	25 g/l

The CSL, MOPS and the salt solution were brought to pH 7 with aqueous ammonia and autoclaved. The sterile substrate and vitamin solutions were then added, as well as the CaCO₃ 5 autoclaved in the dry state.

Culturing was carried out in a 10 ml volume in a 100 ml conical flask with baffles. Kanamycin (25 mg/l) was added. Culturing was carried out at 33°C and 80% atmospheric humidity.

- 10 After 24 and 48 hours, the OD was determined at a measurement wavelength of 660 nm with a Biomek 1000 (Beckmann Instruments GmbH, Munich). The amount of lysine formed was determined with an amino acid analyzer from Eppendorf-BioTronik (Hamburg, Germany) by ion exchange
15 chromatography and post-column derivatization with ninhydrin detection.

The result of the experiment is shown in Table 1.

Table 1

Strain	Time, hours	Lysine-HCl g/l
DSM5715	24	8.1
DSM5715::pSUZ1	24	8.6
DSM5715	48	14.7
DSM5715::pSUZ1	48	15.4

Original (for SUBMISSION) - printed on 03.07.2000 03:06:22 PM

0-1	Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared using	PCT-EASY Version 2.90 (updated 08.03.2000)
0-2	International Application No.	
0-3	Applicant's or agent's file reference	990228 BT
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	18
1-2	line	5-10
1-3	Identification of Deposit	
1-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
1-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
1-3-3	Date of deposit	26 January 2000 (26.01.2000)
1-3-4	Accession Number	DSMZ 13263
1-4	Additional Indications	NONE
1-5	Designated States for Which Indications are Made	all designated States
1-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

FOR RECEIVING OFFICE USE ONLY

0-4	This form was received with the international application: (yes or no)	yes
0-4-1	Authorized officer	C. van Amstel

FOR INTERNATIONAL BUREAU USE ONLY

0-5	This form was received by the international Bureau on:	18.09.2000.
0-5-1	Authorized officer	N. Wagner

Patent claims

1. An isolated polynucleotide from coryneform bacteria,
comprising a polynucleotide sequence chosen from the
group consisting of
 - 5 a) polynucleotide which is identical to the extent of
at least 70 % to a polynucleotide which codes for a
polypeptide which comprises the amino acid
sequences of SEQ ID NO. 2 or SEQ ID NO. 4,
 - 10 b) polynucleotide which codes for a polypeptide which
comprises an amino acid sequence which is identical
to the extent of at least 70 % to the amino acid
sequences of SEQ ID NO. 2 or SEQ ID NO. 4
 - c) polynucleotide which is complementary to the
polynucleotides of a) or b) and
 - 15 d) polynucleotide comprising at least 15 successive
nucleotides of the polynucleotide sequences of a),
b) or c).
2. A polynucleotide as claimed in claim 1
wherein the polynucleotide is a preferably recombinant
20 DNA which is capable of replication in coryneform
bacteria and additionally contains at least one of the
nucleotide sequences which codes for the genes tkt,
zwf, opcA and devB.
3. A polynucleotide as claimed in claim 1,
25 wherein the polynucleotide is an RNA.
4. A polynucleotide as claimed in claim 2,
comprising one of the nucleotide sequence as shown in
SEQ ID NO. 3.
5. A polynucleotide as claimed in claim 2,
30 which codes for a polypeptide which comprises the

amino acid sequence as shown in SEQ ID NO. 2 and SEQ ID NO. 4.

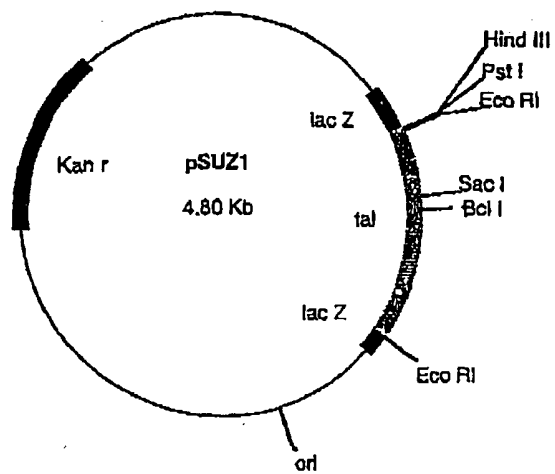
6. A DNA as claimed in claim 2 which is capable of replication, comprising
 - 5 (i) a nucleotide sequence as shown in SEQ ID NO. 3, or
 - (ii) at least one sequence which corresponds to sequences (i) within the range of the degeneration of the genetic code, or
 - 10 (iii) at least one sequence which hybridizes with the sequences complementary to sequences (i) or (ii), and optionally
 - (iv) sense mutations of neutral function in (i).
7. A coryneform bacterium serving as the host cell, which
15 contains a vector which carries a polynucleotide as claimed in claim 1.
8. A process for the preparation of L-amino acids, which comprises carrying out the following steps:
 - 20 a) fermentation of the bacteria which produce the desired L-amino acid, in which at least the tal gene and optionally one or more of the genes tkt gene, zwt gene, devB gene or opcA gene are amplified at the same time,
 - 25 b) concentration of the desired product in the medium or in the cells of the bacteria and
 - c) isolation of the desired L-amino acid.
9. A process as claimed in claim 8, wherein
30 bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally amplified are employed.

10. A process as claimed in claim 8,
wherein
bacteria in which the metabolic pathways which reduce
the formation of the desired L-amino acid are at least
5 partly eliminated are employed.
11. A process as claimed in one or more of claims 8 to 12,
wherein
coryneform bacteria which produce one of the amino
acids from the group consisting of L-lysine, L-
10 threonine, L-isoleucine or L-tryptophan are used.
12. A process for the fermentative preparation of L-amino
acids, in particular lysine, as claimed in claim 8,
wherein
in the coryneform microorganisms which in particular
15 already produce L-amino acids, one or more genes
chosen from the group consisting of
- 12.1 the dapA gene which codes for
dihydrodipicolinate synthase,
- 12.2 the lysC gene which codes for a feed back
20 resistant aspartate kinase,
- 12.3 the gap gene which codes for glycerolaldehyde 3-
phosphate dehydrogenase,
- 12.4 the pyc gene which codes for pyruvate
carboxylase,
- 25 12.5 the mqo gene which codes for malate-quinone
oxidoreductase,
- 12.6 the tkt gene which codes for transketolase,
- 12.7 the gnd gene which codes for 6-phosphogluconate
dehydrogenase,

- 12.8 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 12.9 the lysE gene which codes for lysine export,
- 12.10 the zwal gene,
- 5 12.11 the eno gene which codes for enolase,
- 12.12 the opcA gene
- is or are amplified or over-expressed at the same time.
13. A process for the fermentative preparation of L-threonine as claimed in claim 8,
10 wherein
in coryneform microorganisms which in particular already produce L-threonine, one or more genes chosen from the group consisting of
- 15 13.1 at the same time the hom gene which codes for homoserine dehydrogenase or the hom^{dr} allele which codes for a "feed back resistant" homoserine dehydrogenase,
- 13.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase,
20
- 13.3 the pyc gene which codes for pyruvate carboxylase,
- 13.4 the mqo gene which codes for malate:quinone oxidoreductase,
- 25 13.5 the tkt gene which codes for transketolase,
- 13.6 the gnd gene which codes for 6-phosphogluconate dehydrogenase,

- 13.7 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 13.8 the thrE gene which codes for threonine export,
- 13.9 the zwa1 gene,
- 5 13.10 the eno gene which codes for enolase,
- 13.11 the opcA gene
- is or are amplified, in particular over-expressed, at the same time.
14. A process as claimed in claim 10,
10 wherein
for the preparation of L-amino acids, in particular L-lysine, L-threonine, L-isoleucine or L-tryptophan, bacteria in which one or more genes chosen from the group consisting of,
- 15 14.1 the pck gene which codes for phosphoenol pyruvate carboxykinase
- 14.2 the pgi gene which codes for glucose 6-phosphate6 isomerase
- 14.3 the poxB gene which codes for pyruvate oxidase or
- 20 14.4 the zwa2 gene
- is or are attenuated at the same time, are fermented.
15. A use of polynucleotide sequences as claimed in claim 1 as hybridization probes for isolation of the cDNA which codes for the tal gene product.
- 25 16. A use of polynucleotide sequences as claimed in claim 1 as hybridization probes for isolation of the cDNA or genes which have a high similarity with the sequence of the tal gene.

Figure 1:



SEQUENCE PROTOCOL

<110> National University of Ireland, Galway
Degussa-Hüls AG

5 <120> New nucleotide sequences which code for the tal gene

<130> 990228BT

10 <140>
<141>

<160> 4

15 <170> PatentIn Ver. 2.1

<210> 1
<211> 6995
<212> DNA

20 <213> Corynebacterium glutamicum

<220>
<221> CDS
<222> (2471)..(3550)

25 <223> tal-Gen

<400> 1
cacatttgaa ccacagttgg ttataaaatg ggttcaacat cactatgggt agaggtggtg 60
30 acgggtcaga ttaagcaaag actactttcg gggtagatca cctttgcca atttgaacca 120
attaacctaa gtcgtagatc tgatcatcgg atctaacgaa aacgaaccaa aactttgggtc 180
ccggtttaac ccaggaagga ttgaccacct tgacgctgtc acctgaactt caggcgctca 240
35 ctgtacgcaa ttacctctct gattggctcg atgtggacac caaggctgta gacactgttc 300
gtgtcctcgc tgcagacgct gtagaaaact gtggctccgg ccaccaggc accgcaatga 360
40 gcctggctcc ccttgcatc accttgtagc agcgggttat gaacgtagat ccacaggaca 420
ccaactgggc aggcggtgac cgcttcgttc tttcttggg ccactcctct ttgaccagtc 480
acatccagct ttacttgggt ggattcggcc ttgagatgga tgacctgaag gctctgcgca 540
45 cctgggattc cttgacccca ggacaccctg agtaccgcca caccaagggc gttgagatca 600
ccactggccc tcttgccag ggtcttgcac ctgcagttgg tatggccatg gctgctcgtc 660
50 gtgagcgtgg cctattcgac ccaaccgctg ctgagggcga atccccattc gaccaccaca 720
tctacgtcat tgcttctgat ggtgacctgc aggaaggtgt cacctctgag gcatcctcca 780
tcgctggcac ccagcagctg ggcaacctca tcgtgttctg ggatgacaac cgcactctcca 840
55 tcgaagacaa cactgagatc gctttcaacg aggacgttgt tgctcgttac aaggcttacg 900
gctggcagac cattgaggtt gaggctggcg aggacgttgc agcaatcgaa gctgcagtg 960
60 ctgaggctaa gaaggacacc aagcgaccta ccttcacccg cgttcgcacc atcatcggt 1020
tcccagctcc aactatgatg aacaccggtg ctgtgcacgg tgctgctctt ggcgcagctg 1080

```

aggttgacgc aaccaagact gagcttggat tcgatcctga ggctcacttc gcgatcgacg 1140
atgaggttat cgctcacacc cgctccctcg cagagcgcgc tgcacagaag aaggctgcat 1200
5 ggcaggtcaa gttcgatgag tgggcagctg ccaaccctga gaacaaggct ctgttcgacg 1260
gcctgaactc ccgtgagctt ccagcgggct acgctgacga gctcccaaca tgggatgcag 1320
atgagaaggg cgtcgcaact cgtaaggctt ccgaggetgc acttcaggca ctgggcaaga 1380
10 cccttcctga gctgtggggc ggttccgctg acctcgacag ttccaacaac accgtgatca 1440
agggctcccc ttcttcggc cctgagtcca tctccaccga gacctggtct gctgagcctt 1500
15 acggccgtaa cctgcacttc ggtatccgtg agcacgctat gggatccatc ctcaacggca 1560
tttccctcca cgggtggcacc cgcccatacg gcggaacctt cctcatcttc tccgactaca 1620
tgctcctgc agttcgtctt gcagctctca tggagaccga cgcttactac gtctggaccc 1680
20 acgactccat cgggtctggc gaagatggcc caaccacca gcctgttgaa accttggtg 1740
cactgcgcgc catcccaggt ctgtccgtcc tgcgtcctgc agatgcgaac gagaccgcc 1800
25 aggcttgggc tgcagcactt gagtacaagg aaggccctaa gggctcttga ctgaccgcc 1860
agaacgttcc tgttctggaa ggcaccaagg agaaggctgc tgaaggcgtt cgccgcggtg 1920
gctacgtcct gggtgagggt tccaaggaaa cccagatgt gatcctcatg ggctccggct 1980
30 ccgaggttca gcttgcagtt aacgtgcga aggctctgga agctgagggc gttgcagctc 2040
gcgtgtttc cgttccttgc atggattggt tccaggagca ggacgcagag tacatcgagt 2100
35 ccgttctgcc tgcagctgtg accgctcgtg tgtctgttga agctggcacc gcaatgcctt 2160
ggtaccgctt ctggggcacc cagggccgtg ctgtctccct tgagcacttc ggtgcttctg 2220
cggattacca gacctgttt gagaagttcg gcataccac cgatgcagtc gtggcagcgg 2280
40 ccaaggactc cattaacggt taattgccct gctgttttta gttcaaccg ggggcaatat 2340
gattctccgg aattttattg ccccggttg ttgttgtaa tcggtacaaa gggctcttaag 2400
45 cacatccctt acttgctgc tctccttgag cacagttcaa gaacaattct tttaaggaaa 2460
atthagtttc atg tct cac att gat gat ctt gca cag ctc ggc act tcc 2509
      Met Ser His Ile Asp Asp Leu Ala Gln Leu Gly Thr Ser
          1             5             10
50 act tgg ctc gac gac ctc tcc cgc gag cgc att act tcc ggc aat ctc 2557
      Thr Trp Leu Asp Asp Leu Ser Arg Glu Arg Ile Thr Ser Gly Asn Leu
          15             20             25

```

	agc	cag	gtt	att	gag	gaa	aag	tct	gta	gtc	ggc	gtc	acc	acc	aac	cca	2605
	Ser	Gln	Val	Ile	Glu	Glu	Lys	Ser	Val	Val	Gly	Val	Thr	Thr	Asn	Pro	
	30					35					40					45	
5	gct	att	ttc	gca	gca	gca	atg	tcc	aag	ggc	gat	tcc	tac	gac	gct	cag	2653
	Ala	Ile	Phe	Ala	Ala	Ala	Met	Ser	Lys	Gly	Asp	Ser	Tyr	Asp	Ala	Gln	
					50					55					60		
10	atc	gca	gag	ctc	aag	gcc	gct	ggc	gca	tct	gtt	gac	cag	gct	gtt	tac	2701
	Ile	Ala	Glu	Leu	Lys	Ala	Ala	Gly	Ala	Ser	Val	Asp	Gln	Ala	Val	Tyr	
				65					70					75			
15	gcc	atg	agc	atc	gac	gac	gtt	cgc	aat	gct	tgt	gat	ctg	ttc	acc	ggc	2749
	Ala	Met	Ser	Ile	Asp	Asp	Val	Arg	Asn	Ala	Cys	Asp	Leu	Phe	Thr	Gly	
			80					85					90				
	atc	ttc	gag	tcc	tcc	aac	ggc	tac	gac	ggc	cgc	gtg	tcc	atc	gag	gtt	2797
	Ile	Phe	Glu	Ser	Ser	Asn	Gly	Tyr	Asp	Gly	Arg	Val	Ser	Ile	Glu	Val	
		95					100					105					
20	gac	cca	cgt	atc	tct	gct	gac	cgc	gac	gca	acc	ctg	gct	cag	gcc	aag	2845
	Asp	Pro	Arg	Ile	Ser	Ala	Asp	Arg	Asp	Ala	Thr	Leu	Ala	Gln	Ala	Lys	
	110					115					120					125	
25	gag	ctg	tgg	gca	aag	gtt	gat	cgt	cca	aac	gtc	atg	atc	aag	atc	cct	2893
	Glu	Leu	Trp	Ala	Lys	Val	Asp	Arg	Pro	Asn	Val	Met	Ile	Lys	Ile	Pro	
					130					135					140		
30	gca	acc	cca	ggt	tct	ttg	cca	gca	atc	acc	gac	gct	ttg	gct	gag	ggc	2941
	Ala	Thr	Pro	Gly	Ser	Leu	Pro	Ala	Ile	Thr	Asp	Ala	Leu	Ala	Glu	Gly	
				145					150					155			
	atc	agc	gtt	aac	gtc	acc	ttg	atc	ttc	tcc	gtt	gct	cgc	tac	cgc	gag	2989
	Ile	Ser	Val	Asn	Val	Thr	Leu	Ile	Phe	Ser	Val	Ala	Arg	Tyr	Arg	Glu	
			160					165					170				
	gtc	atc	gct	gcg	ttc	atc	gag	ggc	atc	aag	cag	gct	gct	gca	aac	ggc	3037
	Val	Ile	Ala	Ala	Phe	Ile	Glu	Gly	Ile	Lys	Gln	Ala	Ala	Ala	Asn	Gly	
		175					180					185					
40	cac	gac	gtc	tcc	aag	atc	cac	tct	gtg	gct	tcc	ttc	ttc	gtc	tcc	cgc	3085
	His	Asp	Val	Ser	Lys	Ile	His	Ser	Val	Ala	Ser	Phe	Phe	Val	Ser	Arg	
	190					195					200					205	
45	gtc	gac	gtt	gag	atc	gac	aag	cgc	ctc	gag	gca	atc	gga	tcc	gat	gag	3133
	Val	Asp	Val	Glu	Ile	Asp	Lys	Arg	Leu	Glu	Ala	Ile	Gly	Ser	Asp	Glu	
					210					215					220		
50	gct	ttg	gct	ctg	cgc	ggc	aag	gca	ggc	gtt	gcc	aac	gct	cag	cgc	gct	3181
	Ala	Leu	Ala	Leu	Arg	Gly	Lys	Ala	Gly	Val	Ala	Asn	Ala	Gln	Arg	Ala	
				225					230					235			
	tac	gct	gtg	tac	aag	gag	ctt	ttc	gac	gcc	gcc	gag	ctg	cct	gaa	ggt	3229
	Tyr	Ala	Val	Tyr	Lys	Glu	Leu	Phe	Asp	Ala	Ala	Glu	Leu	Pro	Glu	Gly	
		240						245					250				
55	gcc	aac	act	cag	cgc	cca	ctg	tgg	gca	tcc	acc	ggc	gtg	aag	aac	cct	3277
	Ala	Asn	Thr	Gln	Arg	Pro	Leu	Trp	Ala	Ser	Thr	Gly	Val	Lys	Asn	Pro	
		255					260					265					
60	gcg	tac	gct	gca	act	ctt	tac	gtt	tcc	gag	ctg	gct	ggt	cca	aac	acc	3325
	Ala	Tyr	Ala	Ala	Thr	Leu	Tyr	Val	Ser	Glu	Leu	Ala	Gly	Pro	Asn	Thr	
	270					275					280					285	

gtc aac acc atg cca gaa ggc acc atc gac gcg gtt ctg gag cag ggc 3373
 Val Asn Thr Met Pro Glu Gly Thr Ile Asp Ala Val Leu Glu Gln Gly
 290 295 300

5 aac ctg cac ggt gac acc ctg tcc aac tcc gcg gca gaa gct gac gct 3421
 Asn Leu His Gly Asp Thr Leu Ser Asn Ser Ala Ala Glu Ala Asp Ala
 305 310 315

10 gtg ttc tcc cag ctt gag gct ctg ggc gtt gac ttg gca gat gtc ttc 3469
 Val Phe Ser Gln Leu Glu Ala Leu Gly Val Asp Leu Ala Asp Val Phe
 320 325 330

15 cag gtc ctg gag acc gag ggt gtg gac aag ttc gtt gct tct tgg agc 3517
 Gln Val Leu Glu Thr Glu Gly Val Asp Lys Phe Val Ala Ser Trp Ser
 335 340 345

20 gaa ctg ctt gag tcc atg gaa gct cgc ctg aag tagaatcagc acgctgcac 3570
 Glu Leu Leu Glu Ser Met Glu Ala Arg Leu Lys
 350 355 360

agtaacggcg acatgaaatc gaattagttc gatcttatgt ggccggtaca catctttcat 3630

25 taaagaaagg atcgtgacac taccatcgtg agcacaaaca cgaccccctc cagctggaca 3690
 aacccactgc gcgaccgcga ggataaacga ctcccccgca tcgctggccc ttccggcatg 3750
 gtgatcttcg gtgtcactgg cgacttggct cgaaagaagc tgctccccgc catttatgat 3810

30 ctagcaaacc gcggattgct gccccagga ttctcgttgg taggttacgg ccgccgcgaa 3870
 tgggccaaag aagactttga aaaatacgtc cgcgatgccg caagtgcctg tgctcgtacg 3930
 gaattccgtg aaaatgtttg ggagcgctc gccgagggtg tggaatttgt tcgcggaac 3990

35 tttgatgatg atgcagcttt cgacaacctc gctgcaacac tcaagcgcat cgacaaaacc 4050
 cgcggaaccg ccggcaactg ggcttactac ctgtccattc caccagattc cttcacagcg 4110

40 gtctgccacc agctggagcg ttccggcatg gctgaatcca ccgaagaagc atggcgccgc 4170
 gtgatcatcg agaagccttt cggccacaac ctgcaatccg cacacgagct caaccagctg 4230
 gtcaacgcag tcttcccaga atcttctgtg ttccgcatcg accactattt gggcaaggaa 4290

45 acagttcaaa acatcctggc tctgcgtttt gctaaccagc tgtttgagcc actgtggaac 4350
 tccaactacg ttgaccacgt ccagatcacc atggctgaag atattggctt ggggtggacgt 4410

50 gctggttact acgacggcat cggcgagcc cgcgacgtca tccagaacca cctgatccag 4470
 ctcttggtc tggttgccat ggaagaacca atttctttcg tgccagcgca gctgcaggca 4530
 gaaaagatca aggtgctctc tgcgacaaag ccgtgctacc cattggataa aacctccgct 4590

55 cgtggtcagt acgctgccg ttggcagggc tctgagttag tcaagggact tcgcaagaa 4650
 gatggcttca accctgagtc caccactgag acttttgcgg cttgtacctt agagatcacg 4710

60 tctcgtcgtt gggctggtgt gccgttctac ctgcgcaccg gtaagcgtct tggtcgccgt 4770
 gttactgaga ttgccgtggt gtttaagac gcaccacacc agcctttcga cggcgacatg 4830

actgtatccc ttggccaaaa cgccatcgtg attcgcgtgc agcctgatga aggtgtgctc 4890
atccgcttcg gttccaaggt tccaggttct gccatggaag tccgtgacgt caacatggac 4950
5 ttctcctact cagaatcctt cactgaagaa tcacctgaag catacgagcg cctcattttg 5010
gatgcgctgt tagatgaatc cagcctcttc cctaccaacg aggaagtgga actgagctgg 5070
aagattcttg atccaattct tgaagcatgg gatgccgatg gagaaccaga ggattacca 5130
10 gcgggtacgt ggggtccaaa gagcgtgat gaaatgcttt cccgcaacg tccacactgg 5190
cgcaggccat aatttagggg caaaaaatga tctttgaact tccggatacc accaccagc 5250
15 aaatttccaa gaccctaact cgactgcgtg aatcgggcac ccaggtcacc accggccgag 5310
tgctcaccct catcgtggtc actgactccg aaagcgatgt cgctgcagtt accgagtcca 5370
ccaatgaagc ctgcgcgag caccatctc gcgtgatcat ttggtggtt ggcgataaaa 5430
20 ctgcagaaaa caaagttgac gcagaagtcc gtatcggtgg cgacgctggt gcttccgaga 5490
tgatcatcat gcatctcaac ggacctgtcg ctgacaagct ccagtatgtc gtcacaccac 5550
25 tgttgcttcc tgacaccccc atcgttgctt ggtggccagg tgaatcacca aagaatcctt 5610
cccaggaccc aattggacgc atcgcacac gacgcatcac tgatgctttg tacgaccgtg 5670
atgacgcact agaagatcgt gttgagaact atcaccagg tgataccgac atgacgtggg 5730
30 cgcgccctac ccagtggcgg ggacttggtt cctcctcatt ggatcaccca ccacacagcg 5790
aaatcacttc cgtgaggctg accggtgcaa gcggcagtac ctcggtggat ttggctgcag 5850
35 gctggttggc gcggaggctg aaagtgcctg tgatccgcga ggtgacagat gctcccaccg 5910
tgccaaccga tgagtttggg actccactgc tggctatcca gcgcctggag atcgttcgca 5970
ccaccgctc gatcatcatc accatctatg acgctcatac ccttcaggta gagatgccgg 6030
40 aatccggcaa tgccccatcg ctggtggcta ttggtcgtcg aagtgagtcc gactgcttgt 6090
ctgaggagct tcgccacatg gatccagatt tgggtacca gcacgcacta tccggttgt 6150
45 ccagcgtcaa gctggaaacc gtctaaggag aaatacaaca ctatggttga ttagtagcgc 6210
gcacgcgata ctgaagattt ggttgacag gctgcctcca aattcattga ggttgttga 6270
gcagcaactg ccaataatgg caccgcacag gtatggtca ccggtggtgg cgccggcacc 6330
50 aagttgctgg aaaagctcag cgttgatgcg gctgacctg cctgggatcg cattcatgtg 6390
ttcttcggcg atgagcgcaa tgtccctgtc agtgattctg agtccaatga gggccaggct 6450
55 cgtgaggcac tgttgtccaa ggtttctatc cctgaagcca acattcacgg atatggtctc 6510
ggcgacgtag atcttgca ga ggcagccgc gcttacgaag ctgtgttga tgaattcgca 6570
ccaaacggct ttgatcttca cctgctcggc atgggtggcg aaggccatat caactccctg 6630
60 ttccctcaca ccgatgcagt caaggaatcc tccgcaaagg tcatcgcggt gtttgattcc 6690
cctaagcctc cttcagagcg tgcaactcta acccttcctg cggttcactc cgcaaagcgc 6750

gtgtggttgc tggtttctgg tgcggagaag gctgaggcag ctgcggcgat cgtcaacggt 6810
 gagcctgctg ttgagtggcc tgctgctgga gctaccggat ctgaggaaac ggtattgttc 6870
 5 ttggctgatg atgctgcagg aaatctctaa gcagcgccag ctctaacaag aagctttaac 6930
 aagaagctct aacgaaaagc actaacaac taatccgggt gcgaaccttc atctgaatcg 6990
 10 atgga 6995

<210> 2
 <211> 360
 15 <212> PRT
 <213> Corynebacterium glutamicum
 <400> 2
 20 Met Ser His Ile Asp Asp Leu Ala Gln Leu Gly Thr Ser Thr Trp Leu
 1 5 10 15
 Asp Asp Leu Ser Arg Glu Arg Ile Thr Ser Gly Asn Leu Ser Gln Val
 20 25 30
 25 Ile Glu Glu Lys Ser Val Val Gly Val Thr Thr Asn Pro Ala Ile Phe
 35 40 45
 Ala Ala Ala Met Ser Lys Gly Asp Ser Tyr Asp Ala Gln Ile Ala Glu
 50 55 60
 30 Leu Lys Ala Ala Gly Ala Ser Val Asp Gln Ala Val Tyr Ala Met Ser
 65 70 75 80
 35 Ile Asp Asp Val Arg Asn Ala Cys Asp Leu Phe Thr Gly Ile Phe Glu
 85 90 95
 Ser Ser Asn Gly Tyr Asp Gly Arg Val Ser Ile Glu Val Asp Pro Arg
 100 105 110
 40 Ile Ser Ala Asp Arg Asp Ala Thr Leu Ala Gln Ala Lys Glu Leu Trp
 115 120 125
 Ala Lys Val Asp Arg Pro Asn Val Met Ile Lys Ile Pro Ala Thr Pro
 130 135 140
 45 Gly Ser Leu Pro Ala Ile Thr Asp Ala Leu Ala Glu Gly Ile Ser Val
 145 150 155 160
 50 Asn Val Thr Leu Ile Phe Ser Val Ala Arg Tyr Arg Glu Val Ile Ala
 165 170 175
 Ala Phe Ile Glu Gly Ile Lys Gln Ala Ala Ala Asn Gly His Asp Val
 180 185 190
 55 Ser Lys Ile His Ser Val Ala Ser Phe Phe Val Ser Arg Val Asp Val
 195 200 205
 Glu Ile Asp Lys Arg Leu Glu Ala Ile Gly Ser Asp Glu Ala Leu Ala
 210 215 220
 60 Leu Arg Gly Lys Ala Gly Val Ala Asn Ala Gln Arg Ala Tyr Ala Val
 225 230 235 240

Tyr Lys Glu Leu Phe Asp Ala Ala Glu Leu Pro Glu Gly Ala Asn Thr
 245 250 255
 5 Gln Arg Pro Leu Trp Ala Ser Thr Gly Val Lys Asn Pro Ala Tyr Ala
 260 265 270
 Ala Thr Leu Tyr Val Ser Glu Leu Ala Gly Pro Asn Thr Val Asn Thr
 275 280 285
 10 Met Pro Glu Gly Thr Ile Asp Ala Val Leu Glu Gln Gly Asn Leu His
 290 295 300
 Gly Asp Thr Leu Ser Asn Ser Ala Ala Glu Ala Asp Ala Val Phe Ser
 305 310 315 320
 15 Gln Leu Glu Ala Leu Gly Val Asp Leu Ala Asp Val Phe Gln Val Leu
 325 330 335
 20 Glu Thr Glu Gly Val Asp Lys Phe Val Ala Ser Trp Ser Glu Leu Leu
 340 345 350
 Glu Ser Met Glu Ala Arg Leu Lys
 355 360
 25
 <210> 3
 <211> 1083
 <212> DNA
 30 <213> Corynebacterium glutamicum
 <220>
 <221> CDS
 <222> (1)..(1080)
 35 <223> tal
 <400> 3
 atg tct cac att gat gat ctt gca cag ctc ggc act tcc act tgg ctc 48
 Met Ser His Ile Asp Asp Leu Ala Gln Leu Gly Thr Ser Thr Trp Leu
 40 1 5 10 15

	gac gac ctc tcc cgc gag cgc att act tcc ggc aat ctc agc cag gtt	96
	Asp Asp Leu Ser Arg Glu Arg Ile Thr Ser Gly Asn Leu Ser Gln Val	
	20 25 30	
5	att gag gaa aag tct gta gtc ggt gtc acc acc aac cca gct att ttc	144
	Ile Glu Glu Lys Ser Val Val Gly Val Thr Thr Asn Pro Ala Ile Phe	
	35 40 45	
10	gca gca gca atg tcc aag ggc gat tcc tac gac gct cag atc gca gag	192
	Ala Ala Ala Met Ser Lys Gly Asp Ser Tyr Asp Ala Gln Ile Ala Glu	
	50 55 60	
15	ctc aag gcc gct ggc gca tct gtt gac cag gct gtt tac gcc atg agc	240
	Leu Lys Ala Ala Gly Ala Ser Val Asp Gln Ala Val Tyr Ala Met Ser	
	65 70 75 80	
20	atc gac gac gtt cgc aat gct tgt gat ctg ttc acc ggc atc ttc gag	288
	Ile Asp Asp Val Arg Asn Ala Cys Asp Leu Phe Thr Gly Ile Phe Glu	
	85 90 95	
25	tcc tcc aac ggc tac gac ggc cgc gtg tcc atc gag gtt gac cca cgt	336
	Ser Ser Asn Gly Tyr Asp Gly Arg Val Ser Ile Glu Val Asp Pro Arg	
	100 105 110	
30	gca aag gtt gat cgt cca aac gtc atg atc aag atc cct gca acc cca	432
	Ala Lys Val Asp Arg Pro Asn Val Met Ile Lys Ile Pro Ala Thr Pro	
	130 135 140	
35	ggt tct ttg cca gca atc acc gac gct ttg gct gag ggc atc agc gtt	480
	Gly Ser Leu Pro Ala Ile Thr Asp Ala Leu Ala Glu Gly Ile Ser Val	
	145 150 155 160	
40	aac gtc acc ttg atc ttc tcc gtt gct cgc tac cgc gag gtc atc gct	528
	Asn Val Thr Leu Ile Phe Ser Val Ala Arg Tyr Arg Glu Val Ile Ala	
	165 170 175	
45	gcg ttc atc gag ggc atc aag cag gct gct gca aac ggc cac gac gtc	576
	Ala Phe Ile Glu Gly Ile Lys Gln Ala Ala Ala Asn Gly His Asp Val	
	180 185 190	
50	tcc aag atc cac tct gtg gct tcc ttc ttc gtc tcc cgc gtc gac gtt	624
	Ser Lys Ile His Ser Val Ala Ser Phe Phe Val Ser Arg Val Asp Val	
	195 200 205	
55	gag atc gac aag cgc ctc gag gca atc gga tcc gat gag gct ttg gct	672
	Glu Ile Asp Lys Arg Leu Glu Ala Ile Gly Ser Asp Glu Ala Leu Ala	
	210 215 220	
60	ctg cgc ggc aag gca ggc gtt gcc aac gct cag cgc gct tac gct gtg	720
	Leu Arg Gly Lys Ala Gly Val Ala Asn Ala Gln Arg Ala Tyr Ala Val	
	225 230 235 240	
65	tac aag gag ctt ttc gac gcc gcc gag ctg cct gaa ggt gcc aac act	768
	Tyr Lys Glu Leu Phe Asp Ala Ala Glu Leu Pro Glu Gly Ala Asn Thr	
	245 250 255	
70	cag cgc cca ctg tgg gca tcc acc ggc gtg aag aac cct gcg tac gct	816
	Gln Arg Pro Leu Trp Ala Ser Thr Gly Val Lys Asn Pro Ala Tyr Ala	
	260 265 270	

gca act ctt tac gtt tcc gag ctg gct ggt cca aac acc gtc aac acc 864
 Ala Thr Leu Tyr Val Ser Glu Leu Ala Gly Pro Asn Thr Val Asn Thr
 275 280 285

5 atg cca gaa ggc acc atc gac gcg gtt ctg gag cag ggc aac ctg cac 912
 Met Pro Glu Gly Thr Ile Asp Ala Val Leu Glu Gln Gly Asn Leu His
 290 295 300

10 ggt gac acc ctg tcc aac tcc gcg gca gaa gct gac gct gtg ttc tcc 960
 Gly Asp Thr Leu Ser Asn Ser Ala Ala Glu Ala Asp Ala Val Phe Ser
 305 310 315 320

15 cag ctt gag gct ctg ggc gtt gac ttg gca gat gtc ttc cag gtc ctg 1008
 Gln Leu Glu Ala Gly Val Asp Leu Ala Asp Val Phe Gln Val Leu
 325 330 335

20 gag acc gag ggt gtg gac aag ttc gtt gct tct tgg agc gaa ctg ctt 1056
 Glu Thr Glu Gly Val Asp Lys Phe Val Ala Ser Trp Ser Glu Leu Leu
 340 345 350

gag tcc atg gaa gct cgc ctg aag tag 1083
 Glu Ser Met Glu Ala Arg Leu Lys
 355 360

25 <210> 4
 <211> 360
 <212> PRT
 30 <213> Corynebacterium glutamicum

<400> 4
 Met Ser His Ile Asp Asp Leu Ala Gln Leu Gly Thr Ser Thr Trp Leu
 1 5 10 15

35 Asp Asp Leu Ser Arg Glu Arg Ile Thr Ser Gly Asn Leu Ser Gln Val
 20 25 30

40 Ile Glu Glu Lys Ser Val Val Gly Val Thr Thr Asn Pro Ala Ile Phe
 35 40 45

Ala Ala Ala Met Ser Lys Gly Asp Ser Tyr Asp Ala Gln Ile Ala Glu
 50 55 60

45 Leu Lys Ala Ala Gly Ala Ser Val Asp Gln Ala Val Tyr Ala Met Ser
 65 70 75 80

Ile Asp Asp Val Arg Asn Ala Cys Asp Leu Phe Thr Gly Ile Phe Glu
 85 90 95

50 Ser Ser Asn Gly Tyr Asp Gly Arg Val Ser Ile Glu Val Asp Pro Arg
 100 105 110

55 Ile Ser Ala Asp Arg Asp Ala Thr Leu Ala Gln Ala Lys Glu Leu Trp
 115 120 125

Ala Lys Val Asp Arg Pro Asn Val Met Ile Lys Ile Pro Ala Thr Pro
 130 135 140

60 Gly Ser Leu Pro Ala Ile Thr Asp Ala Leu Ala Glu Gly Ile Ser Val
 145 150 155 160

Asn Val Thr Leu Ile Phe Ser Val Ala Arg Tyr Arg Glu Val Ile Ala

	165	170	175
	Ala Phe Ile Glu Gly Ile Lys Gln Ala Ala Ala Asn Gly His Asp Val		
	180	185	190
5	Ser Lys Ile His Ser Val Ala Ser Phe Phe Val Ser Arg Val Asp Val		
	195	200	205
10	Glu Ile Asp Lys Arg Leu Glu Ala Ile Gly Ser Asp Glu Ala Leu Ala		
	210	215	220
	Leu Arg Gly Lys Ala Gly Val Ala Asn Ala Gln Arg Ala Tyr Ala Val		
	225	230	235
15	Tyr Lys Glu Leu Phe Asp Ala Ala Glu Leu Pro Glu Gly Ala Asn Thr		
	245	250	255
	Gln Arg Pro Leu Trp Ala Ser Thr Gly Val Lys Asn Pro Ala Tyr Ala		
	260	265	270
20	Ala Thr Leu Tyr Val Ser Glu Leu Ala Gly Pro Asn Thr Val Asn Thr		
	275	280	285
25	Met Pro Glu Gly Thr Ile Asp Ala Val Leu Glu Gln Gly Asn Leu His		
	290	295	300
	Gly Asp Thr Leu Ser Asn Ser Ala Ala Glu Ala Asp Ala Val Phe Ser		
	305	310	315
30	Gln Leu Glu Ala Leu Gly Val Asp Leu Ala Asp Val Phe Gln Val Leu		
	325	330	335
	Glu Thr Glu Gly Val Asp Lys Phe Val Ala Ser Trp Ser Glu Leu Leu		
	340	345	350
35	Glu Ser Met Glu Ala Arg Leu Lys		
	355	360	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/06304

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54 C12N15/62 C12P13/08 C12Q1/68 C12P13/06
C12P13/22 //(C12P13/08,C12R1:15)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EMBL, EPO-Internal, WPI Data, CHEM ABS Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	UWE KÖHLER ET AL.: "Transaldolase genes from the cyanobacteria <i>Anabaena variabilis</i> and <i>Synechocystis</i> sp. PCC 6803: comparison with other eubacterial and eukaryotic homologues" PLANT MOLECULAR BIOLOGY, vol. 30, 1996, pages 213-218, XP000960916 abstract; figure 1	1,3,6, 15,16
X	JP 09 224661 A (MITSUBISHI CHEM CORP) 2 September 1997 (1997-09-02) sequence listing	1,3,6, 15,16

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

22 November 2000

Date of mailing of the international search report

01/12/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Montero Lopez, B

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/06304

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 9224661 A	02-09-1997	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)